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Adoptive transfer of an autoimmunological labyrinthitis in the guinea pig; animal model for a sympathetic cochleolabyrinthitis

B. GLODDEK, M. ROGOWSKI & W. ARNOLD *Hals-Nasen-Ohrenklinik und Poliklinik, Technische Universität, München, Germany*

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SUMMARY

Sensorineural hearing loss is a common problem in the otolaryngologist's practice, with autoimmune disease of the inner ear being one possible cause. The restoration of auditory function in some patients following immunosuppressive therapy has created a desire to define and understand this disease better. Because of the lack of a well defined detection method to identify this entity clinically, this study was undertaken in order to provide an animal model for autoimmune disease of the inner ear. Previous studies with guinea pigs have demonstrated that sensitized lymphocytes from the systemic circulation migrate to the labyrinth during an immune response in the inner ear. The aim of this study was to prove the capacity of sensitized lymphocytes to transfer autoimmune inner ear disease, and to describe the resulting morphological and physiological changes. Therefore two groups of sensitized lymphocytes partially labelled with a radioactive marker from inbred guinea pigs with an immune response within the inner ear were injected into the bloodstream of naive recipient animals. Most of the labelled cells were observed in the apical turn of the experimental cochlea, while only few cells were detectable in the control cochleas. In addition, the absence of otoacoustic emissions and the loss of outer hair cells observed by electron microscopy were interpreted as a sign of damage caused by the provoked immunopathologic mechanism. The results are discussed as a possible model for a sympathetic cochleolabyrinthitis.

Keywords autoimmunological labyrinthitis cell transfer lymphocyte migration sympathetic cochleolabyrinthitis guinea pig

INTRODUCTION

Otolaryngologists have long sought to identify causes of sensorineural hearing loss (SNHL) that might be reversed by medical treatment. One such entity which has been postulated is autoimmune inner ear disease. The potential improvement in auditory function in these patients following immunosuppressive therapy has created a strong interest among clinicians in a better understanding of this disease.

Immune responses are increasingly recognized as playing an important role in both the defence of the labyrinth from infection, and the production of inner ear pathology due to autoimmune processes or coincidental injury. The responses of locally resident and circulating systemic lymphocytes have been shown to be involved in inner ear immune responses, with the endolymphatic sac playing a particularly critical role [1–4].

Correspondence: Dr B. Gloddek, HNO-Klinik und Poliklinik, Klinikum rechts der Isar der Technischen Universität, Ismaninger Strasse 22, 81656 München, Germany.

Acute hearing loss in the normal contralateral ear clinically observed after a temporal bone fracture or viral infection of a previously deaf ear may be caused by an autoimmune mechanism involving recirculating memory cells sensitized against cochlear tissue. A comparable mechanism is seen in the inflammatory reaction of a healthy eye after traumatic destruction of the other eye, and is called sympathetic ophthalmia [5–7]. The lack of well defined detection methods to identify autoimmune processes within the inner ear despite currently practised methods is still not sufficient to allow the postulation of a primary autoimmune disease of the inner ear [8–10]. In addition, the human inner ear is one of the few organs of the body not amenable to diagnostic biopsy. Therefore, there has been great interest in developing experimental models that mimic clinical entities such as sympathetic cochleolabyrinthitis. In previous studies it has been demonstrated that sensitized lymphocytes in the blood stream migrate to the labyrinth during an immune response within the inner ear [11].

The aim of this study was to determine whether sensitized lymphocytes transfer an autoimmune disease in the absence of

an antigenic stimulus, and to record the resulting morphological and functional aspects of this disease.

MATERIALS AND METHODS

Experimental design

The migration of lymphocytes to the inner ear raised in animals with an experimental labyrinthitis was compared with the migration of cells sensitized only to the antigen keyhold limpet haemocyanin (KLH). Emphasis was placed on the percentage of recovery of radioactivity in the inner ear and the cellular infiltrate in the cochlea. These observations were correlated with ultrastructural and electrophysiological changes.

Animals

Inbred strain 13 guinea pigs weighing 300–400 g were used for all experiments. Animals were checked otoscopically, and excluded if they showed any sign of middle ear infection. For all procedures the animals were anaesthetized with a combination of intramuscular ketamin hydrochloride and xylazin hydrochloride.

Antigen

KLH was obtained from Sigma (Munich, Germany) and used in its associated form for intradermal or inner ear immunization.

Immunization history

Donor animals were divided into two groups according to immunization history. Group A was immunized intradermally with 1 mg KLH in Freund's complete adjuvant (FCA) and then boosted 2 weeks later with 1 mg in Freund's incomplete adjuvant (FIA) until high circulating levels of anti-KLH antibodies were detected. In this group, both inner ears were challenged with KLH 1 week after the boost, as described previously [11]. Group B animals received KLH in FCA and 2 weeks later in FIA intradermally, only without inner ear challenge. Seven days after the induction of a labyrinthitis, or after the last KLH boost, the animals were again deeply anaesthetized and 10 ml blood drawn by direct cardiac puncture. The defibrinated blood was diluted twice with Hanks' balanced salt solution (HBSS) and the lymphocytes isolated by centrifugation over Ficoll–Hypaque for 20 min at 400 *g* at room temperature. Cells were washed twice with RPMI 1640 and their viability assessed by trypan blue exclusion. Half of the cell population was incubated at 37°C for 60 min with 50 μCi/ml ^{51}Cr (as sodium chromate; Amersham, Braunschweig, Germany). The cells were washed again twice with cold RPMI 1640 to remove unbound isotope. The other half of the cells did not receive any label.

Cell transfer

Recipient animals were naive guinea pigs without immunization, and were slowly injected by the intracardiac route with approximately 5×10^7 cells in 2 ml NaCl. Group A ($n = 16$) received the cells of donor group A (half of the group with labelled and the other half with unlabelled lymphocytes), and group B ($n = 14$) the cells of donor group B (half of the group with labelled cells and the other half with unlabelled lymphocytes). The animals injected with labelled lymphocytes were killed after 3 days, and the remaining animals were followed for a period of 1–3 months.

Recovery of radioactivity in harvested organs

Three days after the cell transfer the animals were again lethally anaesthetized and their neck lymph nodes, spleen, blood, small intestine, a piece of skin and both temporal bones removed and weighed. The organs were immediately counted in a gamma counter and the resulting counts expressed as the percentage recovery of total injected radioactivity per organ.

Histology and autoradiography

The cochleas were fixed overnight in 10% formalin with 2% acetic acid and decalcified in buffered 5% EDTA at room temperature for 2 weeks, paraffin embedded and sectioned with a microtome. After deparaffination the slides were dipped in NTB-2 emulsion (Kodak, Rochester, NY) under safelight conditions and kept in the dark for 3 weeks of exposure. The slides were then developed with Dektol (Kodak) for 2 min and fixed in Kodak rapid fix for 5 min, washed for 20 min, and airdried and counterstained with haematoxylin and eosin (H&E).

Electrophysiological examination

Hearing tests were performed with the animal group receiving unlabelled cells before and 1–3 months after the cell transfer. Transitory evoked otoacoustic emissions (TEOAE) were measured employing the ILO 88 system as developed by Bray & Kemp [12].

Scanning electron microscopy

The cochleas of the animal group receiving unlabelled cells were examined by electron microscopy 1–3 months after the transfer. The tissues were fixed by perilymphatic perfusion with 3% glutaraldehyde in 0·1 M sodium-cacodylate-HCl buffer. After removal of the cochleas the tissues were kept in the fixative for 24 h and further processed according to the OTOTO procedure as described by Malick & Wilson [13]. The samples were examined using the scanning electron microscope Philips SEM 505.

Statistical analysis

The Wilcoxon test was employed for statistical analysis.

RESULTS

Recovery of ^{51}Cr label in harvested organs

The recovery of label in immune organs compared with different immunization histories is presented in Fig. 1. Most of the label was trapped in the spleen (0·68–0·75%) regardless of group. Neck lymph nodes exhibited a group-independent four-fold higher activity (0·09%) than the random activity in the control organs (skin and small intestine, 0·02%). All cochleas of the experimental group A showed a three times higher activity than from the control group B ($P < 0·01$). No significant difference was observed between the left and right ear.

Histological findings

On the light microscopic level 13 of 16 cochleas from the experimental group A exhibited a cellular infiltration by neutrophil granulocytes and lymphocytes, especially in the scala tympani. However, in only one ear of 14 in the control group B was a similar finding observed. This ear was also

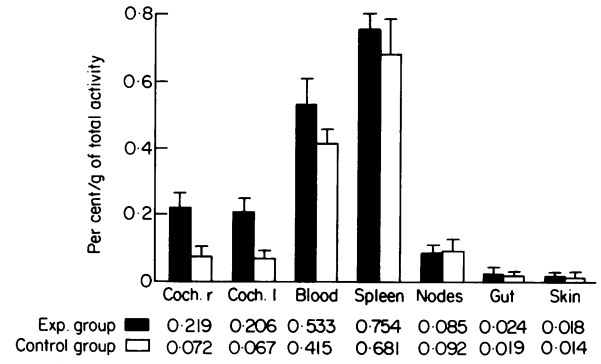

Fig. 1. Recovery of total radioactivity in harvested organs as percentage per gram of total input. Exp. group, experimental group A; coch.r/l, cochlea right/left; nodes, neck nodes.

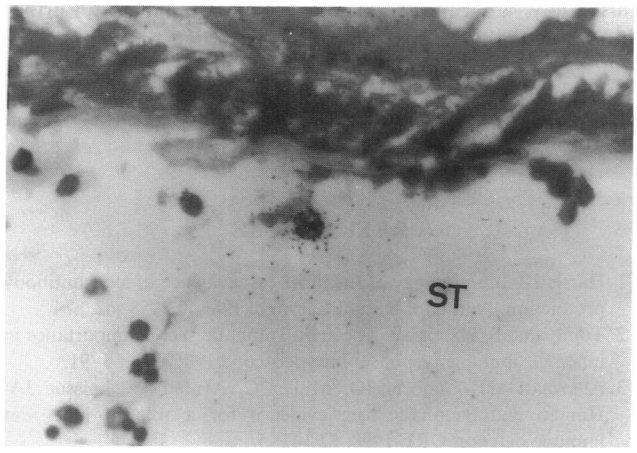

Fig. 2. Radioactive labelled cell in the scala tympani (ST) of the experimental group A (×300).

affected by otitis media. Most labelled cells were detected in the scala tympani in the apical turn of the cochlea and around the modiolar vein (Fig. 2). No marked cells were seen in the control

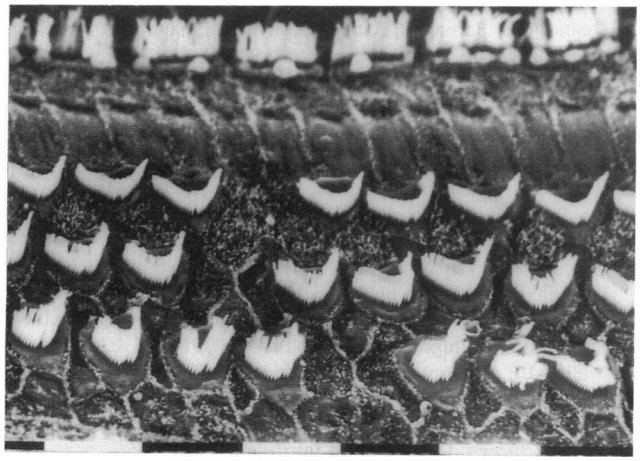

Fig. 3. Surface view of the organ of Corti in scanning electron microscopy (SEM). Loss of outer hair cells in the apical turn 1 month after the cell transfer (experimental group). Bar = 0·1 mm.

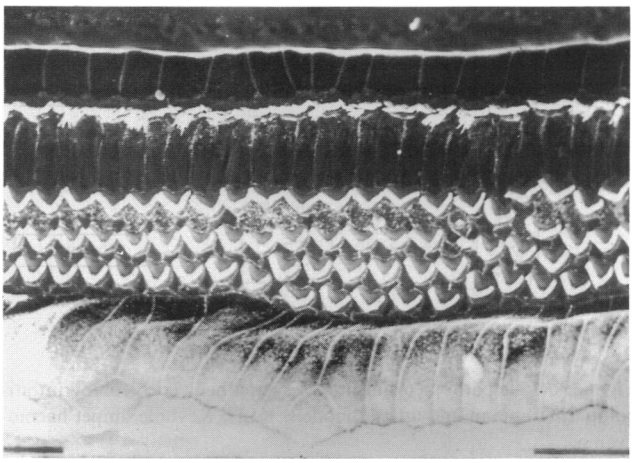

Fig. 4. Normal organ of Corti in SEM of a control group.

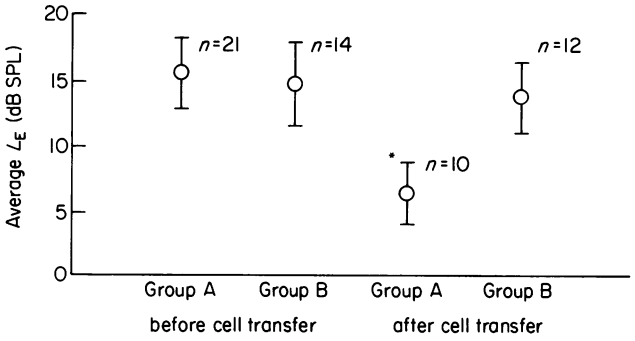

Fig. 5. Synopsis of transitory evoked otoacoustic emission (TEOAE) findings in the experimental group A and control group B before and 1 month after cell transfer. L_E (dBSPL), average sound pressure level of evoked emissions. $^*P < 0{\cdot}01$.

cochleas or in the extravascular compartment of the control organs.

Scanning electron microscopic results were in close correlation to the described light microscopic changes. Frequent loss of outer hair cells (OHC) was detected in the apical turn of the cochlea (Fig. 3), while OHC in the control group remained normal (Fig. 4). This loss was most evident in the third row of the OHC.

Electrophysiologically TEOAE were obtained before the cell transfer in 35 of 36 examined ears in groups A and B. The average values of the sound pressure level (SPL) of the evoked emissions were, in group A 16·2 ± 2·4 dBSPL, and in group B 15·1 ± 3·1 dBSPL. One month after the cell transfer TEOAE could be detected in only 10 ears of group A (48%), while the emissions of the control group B remained in 12 ears (86%). The average value of the emissions dropped in group A to 5·8 ± 2·2 dBSPL, and in group B to 13·9 ± 2·8 dBSPL ($P < 0{\cdot}01$ for group A) (Fig. 5).

DISCUSSION

Previous studies with a similar experimental design were able to demonstrate the origin of infiltrating lymphocytes in the inner ear during an immune response [11]. Most of these cells

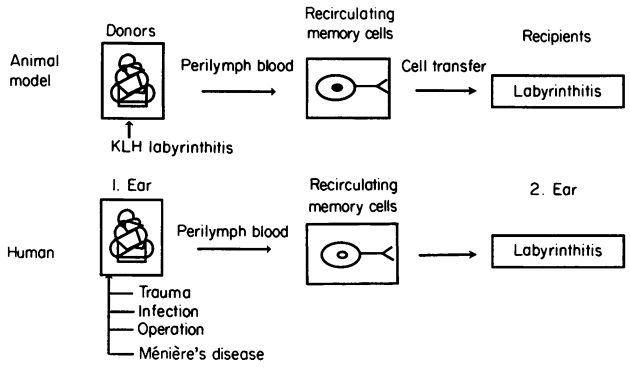

Fig. 6. Possible pathomechanism of a sympathetic cochleolabyrinthitis comparing human and animal models. KLH, Keyhole limpet haemocyanin.

originated in the bloodstream, indicating that the inner ear comes under the immunosurveillance of the systemic circulation during an immune response.

This study was performed to establish the ability of sensitized lymphocytes to transfer an adoptive labyrinthitis in naive animals without antigenic stimulation. The results clearly demonstrated that lymphocytes of donor animals suffering from a labyrinthitis were capable of transferring this organ-specific disease to untreated recipient animals. Time course studies were able to demonstrate that the peak of immigration of cells into the cochlea occurred 3 days after the cell transfer [11]. In contrast, guinea pigs with donor cells raised in animals only immunized to KLH intradermally did not develop a labyrinthitis, the one exception being due to an otitis media with a spread to the inner ear, as previously mentioned [14]. The recovery of label in the blood of the experimental group was significantly higher than from the control group, possibly due to the third antigen challenge as opposed to two in the control group. This might lead to the induction of more memory cells with a prolonged recirculation time in the blood. The observed migration of labelled lymphocytes into the cochlea corresponded to damaging effects on the inner ear. Most of the immigrating cells were found in the scala tympani adjacent to the posterior spiral modiolar vein, which is a known location for cells leaving the blood and entering the cochlea [11]. The damaging effects caused by these lymphocytes were demonstrated morphologically by the loss of hair cells, and physiologically by the inability to record otoacoustic emissions and the decreased average level of the emissions after cell transfer, together indicating the first stage of a labyrinthitis caused by autoreactive lymphocytes. There is a close correlation between the number of labelled cells in the cochlea, the loss of hair cells and the inability/decreased level to record the otoacoustic emissions. The use of otoacoustic emissions is considered a very sensitive technique for detection of the early phase of hearing disorders, and especially reflects the function of the outer hair cells [15].

This experimental design and the resulting histological changes could also be used as an animal model for a sympathetic cochleolabyrinthitis, and as an explanation for organ-specific autoimmune disease of the inner ear. The experimental approach imitates the sensitization of lymphocytes, for example after infection, trauma or operation of the cochlea on one side and subsequent disease of the contralateral ear as clinically observed in humans [16,17]. An autoimmunological etiology has been established for sympathetic ophthalmia [5–7], and is possibly the cause of contralateral labyrinthitis as well. However, the initiating event in sympathetic ophthalmia is traumatic destruction of the eye, while in this study inflammation was used to induce autoreactive memory cells. The common feature of these phenomena may be the release of sequestered proteins into the bloodstream, resulting in the following pathomechanism (Fig. 6): during infection, trauma or operative manipulation of the inner ear, lymphocytes become sensitized as a result of exposure to proteins from the damaged cochlea. These normally anatomically sequestered proteins are recognized as 'foreign' and serve as an antigen, resulting in the induction of sensitized lymphocytes. These cells recirculate as 'memory' lymphocytes (in this experiment the donor cells) and reach the intact contralateral cochlea. This results in an immune response followed by destruction of the organ (in this study labyrinthitis in the recipient animals). Further studies with this animal model will examine the lymphocyte subtypes which transfer this disease process and the role of adhesion molecules and endothelial cells in the entry of lymphocytes into the inner ear [18].

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